Heterogeneity in two herpes simplex virus genes among clinical isolates shows no apparent correlation with neurovirulence of U.S. isolates but may identify a geographic cluster of South African isolates.

Tina Dillas and Gary Lindquester
Department of Biology, Rhodes College

Introduction

Herpes Simplex Virus 1 (HSV-1) belongs to a large family of herpesviruses that have been studied extensively due to their role in human disease. HSV-1 is most commonly associated with cold sores. The virus infects epithelial cells around the mouth resulting in facial lesions. Following primary infection, HSV-1 replicates and spreads to the host nervous system where it establishes latency. The virus can be reactivated by a variety of stimuli that allow it to travel back down the neural axon and into skin tissue where cells are re-infected. HSV-1 infection normally causes mild symptoms; however, in rare cases the virus infects the central nervous system and, if untreated, can lead to fatal encephalitis. This capacity is referred to as neurovirulence.

Herpes simplex encephalitis occurs in approximately 1 out of every 200,000 people infected with HSV-1 each year (Olson et al. 1964). If left untreated, this disease is often fatal. Treatment with antiviral acyclovir has been proven to be effective; however, residual brain damage may be permanent (Whitley & Kimberlin, 2005). HSV-1 neurovirulence allows the virus to manifest into a variety of life threatening conditions. Understanding how the virus moves from its initial site of infection to the nervous system is an important goal in research. By using the mouse as an animal model, researchers have discovered viral genetic factors associated with HSV-1 neurovirulence in a variety of laboratory-derived strains. However, a systematic study seeking correlations between mutations in neurovirulence-associated genes in clinical isolates and relative neurovirulence in the animal model is lacking.

To this end, our laboratory has acquired eighteen clinical isolates of HSV-1 of U.S. and South African origin. The goal for this study is to focus on genetic heterogeneity of two genes, UL39 and UL53, that have been associated with neurovirulence. (Cameron et al. 1988, Matundan et al. 2015). UL39 encodes ribonucleotide reductase that controls apoptosis in epithelial cells (Cameron et al. 1988). UL53 encodes glycoprotein K (gK), an envelope protein involved in virion-host entry (Matundan et al. 2015). DNA sequences were analyzed from the eighteen clinical isolates and two laboratory strains of HSV-1 in order to identify whether genetic variation in UL39 and UL53 correlates to neurovirulence. Such a correlation was not identified; however, genetic variation was associated with geographic origin of strains.

Methods

Twenty HSV-1 strains were acquired from the Centers for Disease Control, including two laboratory reference strains (Justin and HFEM). US clinical isolatesH101, 24, 47, 49, 71,72, 57, 84 were obtained courtesy of Felicia Stamey. South African isolates Spu1, Spu9, Spu372 were obtained from patients with fulminant HSV-1 infection, while D253, D288, D529, D531, and D362 were obtained from patients with typical HSV-1 presentation (courtesy of Robert Swanepoel).

Viral template DNA was prepared from dilutions of purified viral DNA generated in a previous study or from 100 microliters of high titer viral stock eluted with the QIAamp DNA Blood Kit (Qiagen). PCR amplification of the UL39 and UL53 genes was conducted by standard protocols and confirmed by gel electrophoresis. Amplified DNA used for sequencing template was purified with the QIAquick PCR Purification Kit (Qiagen) following manufacturers instructions using the PCR reaction product directly or using supernatant obtained by centrifugation of excised agarose gel bands (Sun et al. 2012). DNA concentrations were determined using Nanodrop Lite. Sequencing at the UT Health Science Center Molecular Resource Center. Raw sequence data were assembled into contigs and aligned using the DNASTAR Lasergene applications as were translated

Results

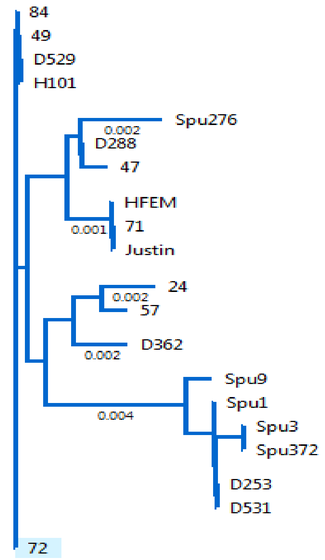

Figure 1: Phylogenetic tree of DNA alignment based on nucleotide region coding of the last 412 amino acids of UL39 gene product

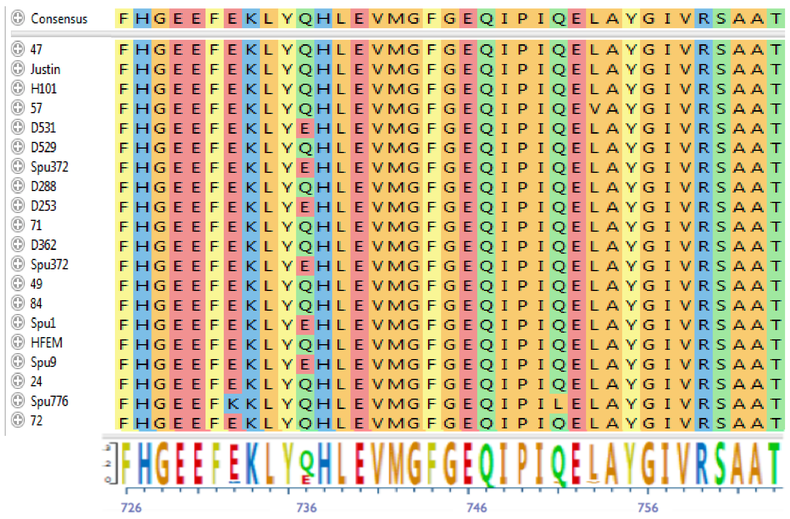

Figure 2: Amino acid alignment of UL39 coding sequence shows variation in position 736 across twenty strains of HSV-1**.** Sequence data were obtained from last 412 amino acids in the UL39 coding sequence corresponding to positions 726- 1137.

Table 1:Catalogue of amino acid variation in the complete UL53 (gK) coding sequence and the last 412 amino acids in the UL39 coding sequence.

UL53 (gK)			UL39		
Strain	**Variant**	**Nature of change**	**Strain**	**Variant**	**Nature of change**
H101, D529	A51T	Nonpolar to polar	Spu276	E732K	Acidic to basic
24	G167C	Nonpolar to polar	D531, Spu372, D253, Spu3, Spu1, Spu9	Q736E	Neutral polar to acidic
Justin, 71, HFEM, 72	F226V	Conserved nonpolar	57	L750V	Conserved nonpolar
D531	P286S	Non polar to polar (Structural)	57	A885V	No change
Spu9	L304I	Conserved polar			

Discussion

Amino acids were aligned and analyzed, and although there are additional nucleotide changes, we focused on the amino acids differences among strains (Table 1). For the gK gene, there are no apparent correlations between amino acid variants and the known neurovirulence (not shown) of the U.S. strains (as presented in Figure 2). The most variable region of the UL39 gene product is shown in Figure 1 with Spu372, Spu 3, Spu1, and Spu9 strains having a Q to E change at position 736. This difference causes the strains to cluster in the phylogenetic analysis (Figure 2). Since the Spu strains were isolated from patients with severe disseminated disease, it is possible that this particular change relates to the severity of disease. However, this cannot be certain as D531 and D523 also exhibited this amino acid change. It is also possible that these strains cluster together because they represent a clade of African origin, although that does not explain why Spu276, D529, D288, and D362 do not fall into this cluster. Further studies will be necessary to address these hypotheses.

References

- Cameron JM, McDougall I, Marsden HS, Preston VG, Ryan DM, Subak-Sharpe JH. (1988) Ribonucleotide reductase encoded by herpes simplex virus is a determinant of the pathogenicity of the virus in mice and a valid antiviral target. J Gen Virol, 69(10):2607-12. Doi: 10.1099/0022-1317-69-10-2607
- Matundan HH, Mott KR, Akhtar AA, Breunig JJ, Ghiasi H. (2015). Mutations within the pathogenic region of herpes simplex virus 1 gK signal sequences alter cell surface expression and neurovirulence. J Virol, 89(5):2530-42. Doi: 10.1128/JVI.03506-14. J Gen Virol. 1988 Oct;69 (Pt 10):2607-12.
- Nahmias AJ, Roizman B. (1973) Infection with Herpes-Simplex Viruses 1 and 2. N Engl J Med, 289:781-789 Doi: 10.1056/NEJM197310112891505
- Olson LC, Buescher EL, Artenstein MS, Parkman PD. (1967). Herpesvirus infections of the human central nervous system.N Engl J Med, 277(24):1271-7. Doi: 10.1056/NEJM196712142772401
- Sun Y, Sriramajayam K, Luo D, Liao DJ. A Quick, Cost-Free Method of Purification of DNA Fragments from Agarose Gel. J Cancer 2012; 3:93-95. Doi:10.7150/jca.4163.
- Whitley RJ, Kimberlin DW. (2005). Herpes simplex encephalitis: children and adolescents. Semin Pediatr Infect Dis, 16(1):17-23. Doi: 10.1053/j.spid.2004.09.007